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Circular-Dichroism and Electron-Microscopy Studies of Human Subcomponent C1q before and after Limited Proteolysis by Pepsin

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1. A fragment of human subcomponent C1q was prepared by limited proteolysis with pepsin at 37°C for 20h, and at pH4.4, followed by gel filtration on Sephadex G-200. This fragment was shown to contain all the collagen-like features known to be present in the intact molecule [Reid (1976) *Biochem. J.* **155**, 5–17]. 2. Circular-dichroism studies showed the presence of positive bands at 230 and 223 nm in the intact subcomponent C1q and pepsin fragment respectively, compared with a positive band at 220 nm obtained for lathyritic rat skin collagen. These bands were abolished by collagenase treatment, which suggested that there may be some collagen-like triple-helical structure in subcomponent C1q and that this structure resides in the pepsin-resistant portion of the molecule. However, the 230 and 223 nm bands had a substantially lower magnitude than that obtained for the unaggregated single fibres of totally triple-helical collagen. 3. Thermal-transition temperatures obtained for subcomponent C1q, the pepsin fragment and the reduced and alkylated pepsin fragment were 48°, 48° and 39°C respectively, compared with a value of 38°C obtained for lathyritic rat skin collagen. 4. Only the unreduced pepsin fragment regained significant amounts (up to 60%) of collagen-like structure, after heat denaturation and cooling, as estimated by circular-dichroism measurements. 5. Electron-microscopy studies of subcomponent C1q and the collagen-like pepsin-resistant fragment of subcomponent C1q showed that the six peripheral globular regions of the molecule were fragmented by pepsin leaving the six collagen-like connecting strands and fibril-like central portion intact.

Human subcomponent C1q is known to have 40% of its amino acid sequence in the form of three different collagen-like regions each 78 amino acid residues long and each composed of the repeating triplet Gly-X-Y, where X is often proline and Y is often hydroxyproline or hydroxylysine (Reid *et al.*, 1972; Reid, 1974, 1976). The imino acid contents of these three collagen-like regions are 14, 23 and 26% (Reid, 1976), which is of the same order as that found in vertebrate collagen partial sequences (18–27%; Dayhoff, 1972). The regions of collagen-like sequence in the native subcomponent C1q are readily fragmented by collagenase treatment (Reid *et al.*, 1972; Lowe & Reid, 1974), but survive pepsin treatment at pH4.4 (Reid, 1976). In their sensitivity to collagenase and resistance to pepsin the collagen-like regions of subcomponent C1q show a remarkable similarity in behaviour to the triple-helical regions of procollagen and collagen. Electron-microscopy studies of subcomponent C1q (Shelton *et al.*, 1972; Knobel *et al.*, 1975) have shown that there are regions in the molecule that have dimensions similar to that expected for a short section of collagen triple helix and another region which could be envisaged as being a fibril formed by the aggregation of the triple-helical regions in the molecule. In view of these observations, which suggested that there might be triple-helical structure in subcomponent C1q, c.d. (circular-dichroism) studies were performed on subcomponent C1q and the pepsin-resistant portion of subcomponent C1q and the results compared with those obtained for lathyritic rat skin collagen. Electron-microscopy studies were also performed since all collagen triple helices examined so far conform to exact and predictable measurements for known lengths of collagen-type amino acid sequence (Traub & Piez, 1971).

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Materials and Methods

Circular-dichroism measurements

The c.d. spectra were recorded on a Roussel-Jouan Dichrograph II instrument, and cells of 1 mm

path-length were used. The temperature of the sample was determined by use of a thermistor which was inserted in the sample cell and attached to a galvanometer. Samples were prepared for c.d. measurements usually by dialysis for 20h against the solvent used in the measurements and then spun before use. The solvents used in the measurements were 10mM-acetic acid, 150mM-KF, pH6.6, and 10mM-Tris/HCl/150mM-NaCl/5mM-$CaCl_2$, pH7.4. Concentrations of samples were in the range 0.5–2.5mg/ml and were determined from the extinction coefficient $E^{1\%}_{1cm} = 6.82$ at 280nm for subcomponent C1q (Reid *et al.*, 1972) and by weight for the pepsin-treated C1q and lathyritic rat skin collagen. C.d. measurements were made from 190 to 260nm. In experiments in which enzyme was added, the c.d. spectrum of the sample was obtained by subtracting the c.d. spectrum of the enzyme from that of the mixture, but it was felt that this could only be done accurately for values above 215nm in view of the relatively large amounts of enzyme used. The measurements made to obtain the thermal-transition temperatures were done in the temperature range 25–70°C, and each sample was measured at its positive extremum at each temperature until equilibrium was reached. The mid-point of the temperature of transition was taken as T_m, and ΔT was taken as the number of degrees between one-quarter and three-quarters thermal transition. All c.d. magnitudes are given as mean residue ellipticities ($[\theta]_{MR}$).

Preparation of human subcomponent C1q, pepsin-treated subcomponent C1q and lathyritic rat skin collagen

Human subcomponent C1q was prepared as described by Reid (1974). The pepsin fragment of subcomponent C1q was isolated by partial pepsin digestion followed by gel filtration on Sephadex G-200 as described by Reid (1976). Collagen samples were obtained by neutral salt extraction of lathyritic rat skins (Piez *et al.*, 1963).

Reduction and alkylation of the pepsin fragment of subcomponent C1q

The pepsin fragment of subcomponent C1q (5mg) was dissolved in 500mM-Tris/HCl/2mM-EDTA, pH8.0, (2.0ml) and reduced by incubation at 37°C for 1h in the presence of 20mM-dithiothreitol. The solution was then made 40mM with respect to recrystallized iodoacetamide and incubated at 37°C for a further 30min. The reduced and alkylated pepsin fragment was then dialysed against several changes of distilled water and finally against 10mM-acetic acid.

Collagenase digestion of subcomponent C1q, the pepsin fragment of subcomponent C1q and lathyritic rat skin collagen

Bacterial collagenase [25μg; CLSPA; Worthington Biochemical Corp., Freehold, NJ, U.S.A.; further purified as described by Peterkofsky & Diegelmann (1971)] in 5μl of the digestion buffer (12.5mM-Tris/HCl/150mM-NaCl/5mM-$CaCl_2$, pH7.4) was added to 160μg of subcomponent C1q, the pepsin fragment of subcomponent C1q or lathyritic rat skin collagen dissolved in 150μl of the digestion buffer. Digestion was carried out at 35°C and was continuously monitored by c.d. at 230, 223 and 220nm for subcomponent C1q, the pepsin fragment and lathyritic rat skin collagen respectively. Digestion was performed, and continuously monitored, for up to 20h for intact subcomponent C1q owing to its low rate of digestion by bacterial collagenase (Reid *et al.*, 1972). Digestion of the pepsin fragment of subcomponent C1q and of lathyritic rat skin collagen was monitored for not more than 60min, since complete digestion, as judged by loss of the positive band in the c.d. spectra, at 223 and 220nm respectively, took not more than 2min under the same conditions as those used for digestion of intact subcomponent C1q.

Electron microscopy of subcomponent C1q and the pepsin fragment of subcomponent C1q

Samples of subcomponent C1q and the pepsin fragment were diluted to a concentration of about 10ng/ml in 300mM-ammonium acetate buffer, pH7.0, and negatively stained with aq. 1% (w/v) uranyl acetate by the method of Valentine *et al.* (1968). The preparations were examined in a Hitachi HU 11 ES electron microscope, operating at 75kV with 200nm condenser aperture and 50nm objective aperture.

Results

C.d. spectra of subcomponent C1q and the pepsin fragment of subcomponent C1q

The c.d. spectra of subcomponent C1q, the pepsin fragment and lathyritic rat skin collagen, all in 10mM-acetic acid, are shown in Fig. 1, and the c.d. extrema of all these samples are given in Table 1. Samples in 150mM-KF, pH6.6, gave very similar results. Subcomponent C1q shows a small maximum near 230nm (+800) and a minimum near 200nm (−5500). After pepsin digestion, the positive band increased in magnitude (+2300) and shifted to a lower wavelength (223nm), and the negative band at 200nm also increased in magnitude (−16 450). Reduction and alkylation of the pepsin fragment did not affect the c.d. spectrum significantly (Table 2).

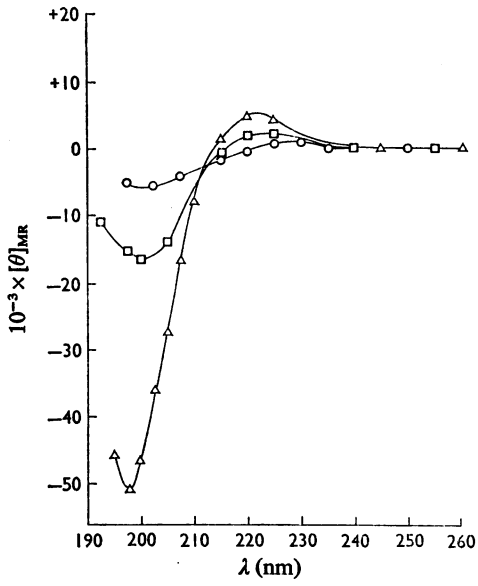


Fig. 1. *C.d. spectra of human subcomponent C1q, the pepsin fragment and lathyritic rat skin collagen*

The c.d. spectra of human subcomponent C1q (○), the pepsin fragment of subcomponent C1q (□) and lathyritic rat skin collagen (△) are shown. All samples were measured at concentrations of 0.5–2.5mg/ml, in 10mM-acetic acid, at room temperature, and the spectra are plotted in mean residue ellipticities.

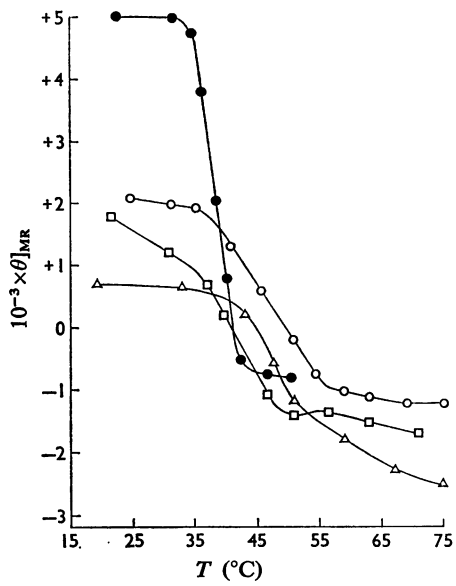


Fig. 2. *'Melting' curves of human subcomponent C1q, the pepsin fragment and lathyritic rat skin collagen*

'Melting' curves of intact human subcomponent C1q (△), the pepsin fragment of subcomponent C1q (○), the reduced and alkylated pepsin fragment of subcomponent C1q (□) and lathyritic rat skin collagen (●) in 10mM-acetic acid were monitored at 230, 223, 223 and 220nm respectively. All samples were examined at a concentration of 0.5–2.5mg/ml. Each sample was measured at its positive extremum at each temperature until equilibration.

Table 1. *C.d. extrema obtained for subcomponent C1q and the collagen-like pepsin fragment of subcomponent C1q*

The c.d. extrema of lathyritic rat skin collagen, subcomponent C1q and the collagen-like pepsin fragment of subcomponent C1q were measured in 10mM-acetic acid at room temperature. Full experimental details are given in the text. Similar values were obtained in 150mM-KF, pH6.6. The magnitudes are given as mean residue ellipticities.

	C.d. extrema			
	Native		Heat-denatured	
Sample	nm	magnitude	nm	magnitude
Lathyritic rat skin collagen	198	−50000	200	−15000
	220	+5000		
Subcomponent C1q	200	−5800	205	−2400
	230	+800		
Pepsin fragment of subcomponent C1q	200	−16450	200	−10490
	223	+2200		
Reduced and alkylated pepsin fragment of subcomponent C1q	200	−8050	200	−4250
	223	+2400		

Thermal-transition curves of subcomponent C1q and the pepsin fragment of subcomponent C1q

The c.d. spectra of subcomponent C1q, the pepsin fragment and lathyritic rat skin collagen in 10mM-acetic acid were recorded at increasing temperatures, and these curves are shown in Fig. 2. The curves suggest 'melting out' of some structure, and the T_m (mid-point of the transition) and ΔT (breadth of the transitions) are listed in Table 2. The T_m of both subcomponent C1q and the pepsin fragment are approx. 47°C, a temperature considerably higher than that of 38°C observed for lathyritic rat skin collagen (Table 2). When subcomponent C1q was heated in 150mM-KF, pH6.6, the band at 230nm first decreased and went negative, and then the protein precipitated out of solution (between 50° and 55°C) (Fig. 3). The pepsin fragment of subcomponent C1q 'melted out' in the same manner in 150mM-KF as it did in 10mM-acetic acid, with no signs of precipitation (Fig. 3).

After the samples were heated for these thermal-transition curves, they were allowed to cool down to room temperature (25°C) overnight, and later placed at 4°C to see if any structure could be regained. The

Table 2. *T_m and ΔT values obtained from 'melting' curves of subcomponent C1q and the collagen-like pepsin fragment of subcomponent C1q*

C.d. measurements, made over a temperature range of 25–70°C, allowed 'melting' curves to be plotted for lathyritic rat skin collagen, subcomponent C1q and the collagen-like pepsin fragment of subcomponent C1q, these samples being monitored at 220, 230 and 223 nm respectively. The mid-point of the temperature transition was taken as T_m, and ΔT was taken as the number of degrees between one-quarter and three-quarters 'melting'.

Sample	T_m (°C)	ΔT (°C)
Lathyritic rat skin collagen	38	3
Subcomponent C1q	48	15
Pepsin fragment of subcomponent C1q	48	8
Reduced and alkylated pepsin-treated C1q	39	11

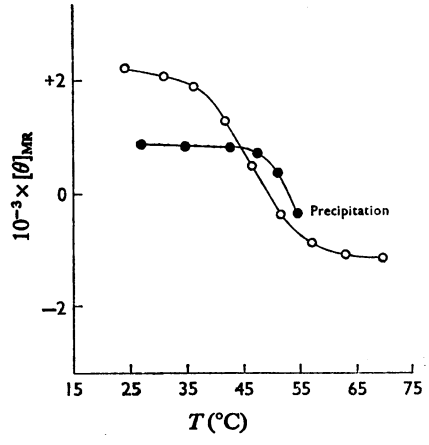


Fig. 3. *'Melting' curve of subcomponent C1q and the pepsin fragment of subcomponent C1q in 150 mM-KF, pH 6.6*

'Melting' curves of human subcomponent C1q (●) and the pepsin fragment of subcomponent C1q (○) in 150 mM-KF, pH 6.6, were monitored at 230 and 223 nm respectively. Both samples were examined at concentrations of 0.5–2.5 mg/ml. Each sample was measured at its positive extremum at each temperature until equilibration.

unreduced sample of the pepsin fragment of subcomponent C1q consistently regained a positive band at 223 nm after such cooling, with a magnitude of, at most, 60% of the original value before 'melting'. Neither the whole subcomponent C1q molecule nor the reduced and alkylated pepsin fragment of subcomponent C1q ever regained a positive band after cooling.

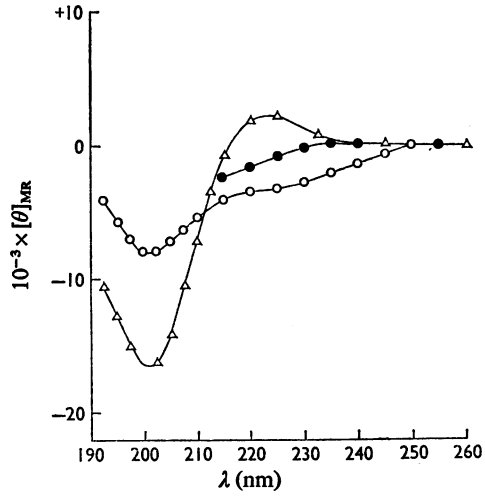


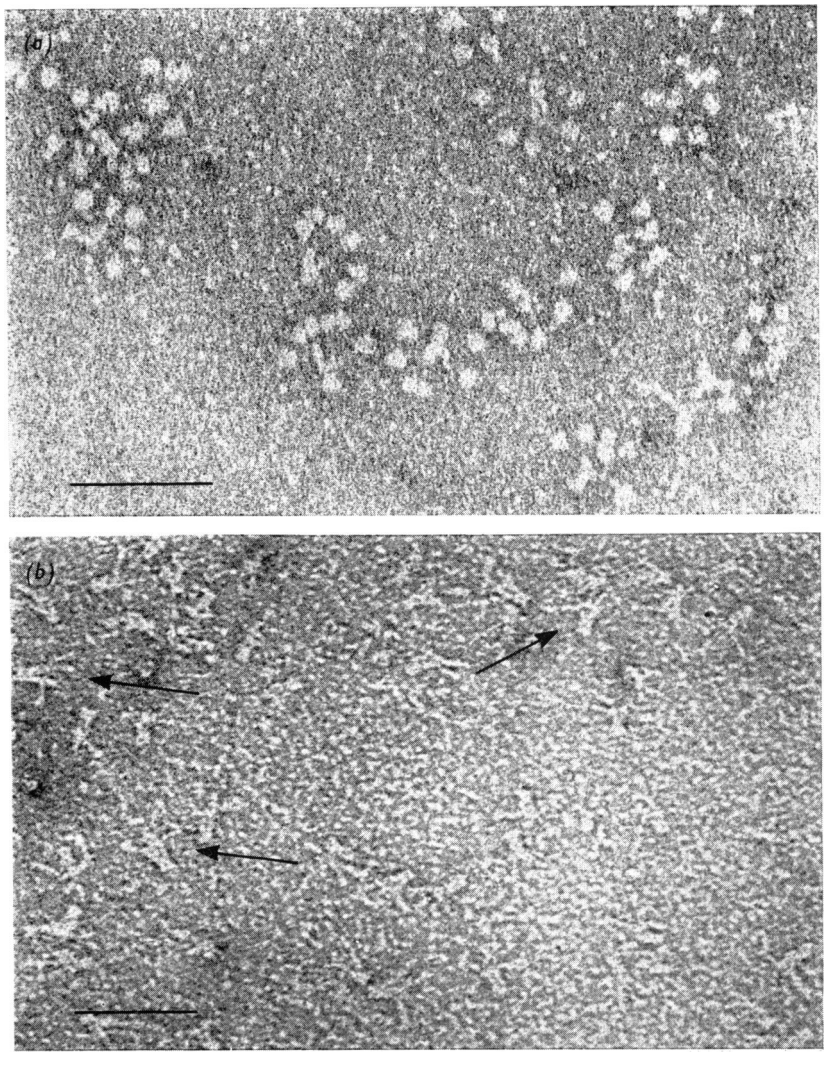
Fig. 4. *C.d. spectra of the pepsin fragment of subcomponent C1q after denaturation by heat or digestion with collagenase*

The c.d. spectra of the native pepsin fragment of subcomponent C1q (△), the heat-denatured fragment of subcomponent C1q (○) and the collagenase-digested pepsin fragment of subcomponent C1q (●) are shown. The first two samples were measured in 10 mM-acetic acid and the third sample in 12.5 mM-Tris/HCl/150 mM-NaCl/5 mM-$CaCl_2$, pH 7.4, and the spectra are plotted in mean residue ellipticities.

Collagenase digestion of native subcomponent C1q, the pepsin fragment of subcomponent C1q, and lathyritic rat skin collagen

Digestion of the pepsin fragment of subcomponent C1q and lathyritic rat skin collagen with collagenase rapidly abolished the positive bands seen at 223 and 220 nm respectively, and after 1 min digestion the c.d. measurements at 223 and 220 nm respectively, were slightly negative (Fig. 4). Digestion of subcomponent C1q with collagenase abolished the positive band seen at 230 nm but, in contrast with the results obtained with the pepsin fragment and the lathyritic rat skin collagen, it took 15–20 h to achieve this and after this time a value of zero was recorded at 230 nm rather than a negative value.

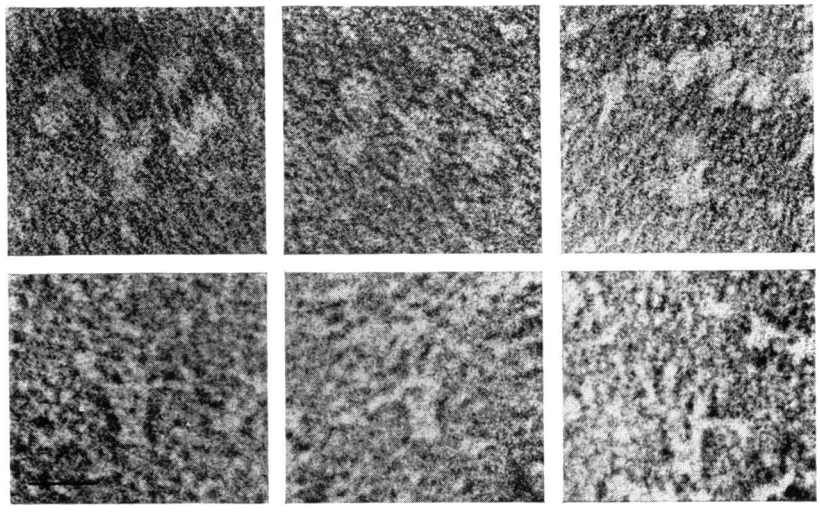
Previous results have shown that the collagen-like regions of subcomponent C1q are diminished to small peptides by collagenase digestion, whereas the non-collagen-like regions are left intact and that the digestion took 15–20 h to reach completion (Reid *et al.*, 1972).



EXPLANATION OF PLATE 1

Whole and pepsin-treated subcomponent C1q

(*a*) Whole subcomponent C1q, negatively stained with 1% (w/v) uranyl acetate. Magnification ×47000. Scale bar represents 50nm. (*b*) Pepsin-treated subcomponent C1q, negatively stained with uranyl acetate. The molecules indicated by arrows are shown at a higher magnification in Plate 2. Magnification ×42000. Scale bar represents 50nm.



EXPLANATION OF PLATE 2

Single molecules of intact subcomponent C1q (upper row) and the pepsin fragment of subcomponent C1q (lower row) shown at the same magnification and the same orientation for comparison

Magnification $\times 1.2 \times 10^6$. Scale bar represents 10nm.

Electron microscopy of subcomponent C1q and the pepsin fragment of subcomponent C1q

Preparations of whole subcomponent C1q showed the structure of six peripheral globular portions linked to a central portion by flexible connecting strands, as has been reported previously (Shelton *et al.*, 1972; Knobel *et al.*, 1975). In regions of negative stain where the stain sheet was thin, the molecules appeared to lie on the supporting carbon fibres sideways on (Plate 1*a*). Although there is probably some distortion of the molecule under these conditions, the peripheral globular portions appeared consistently to be roughly circular in cross section, whereas the central portion appeared more rectangular. In thicker regions of stain the central portion tended to orient itself at right angles to the substrate, with the six peripheral globular portions arranged radially about it (Plates 1*a* and 2).

Preparations of the pepsin fragment of subcomponent C1q were more difficult to detect in the electron microscope. However, images were obtained, which were consistent with the appearance of subcomponent C1q molecules from which the peripheral globular units had been removed, leaving intact the central portion and the flexible connecting strands.

Preparations of subcomponent C1q that had been renatured after heat-treatment were also examined. For the whole subcomponent C1q and the reduced and alkylated pepsin fragment of subcomponent C1q, where the degree of renaturation, as measured by the recovery of the positive bands at 230nm or 223nm in the c.d. spectra, was small, there did not appear to be any recognizable intact whole molecules or intact fragments present. Renatured unreduced pepsin fragment of subcomponent C1q, for which there was approx. 60% recovery of the 223nm band in the c.d. spectrum, showed some rod-like particles, 20–30nm in length and about 2–3nm in width, which may be part of the central portion plus a linear aggregate of

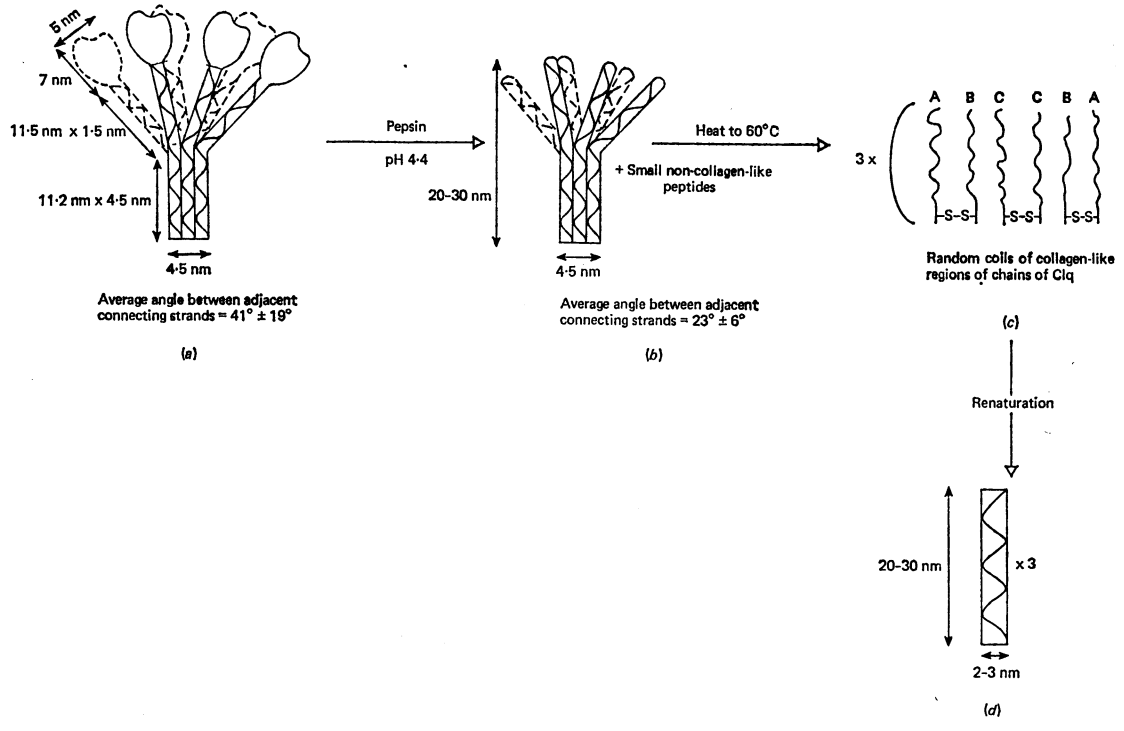


Fig. 5. *Diagrammatic representation of the electron-microscopy and c.d. studies of intact and pepsin-digested subcomponent C1q*

The electron-microscopy measurements for the intact subcomponent C1q have been published previously (Shelton *et al.*, 1972; Knobel *et al.*, 1975). ——, Portions of the molecule pointing towards the reader; – – – –, portions of the molecule pointing inward, away from reader; ∼, and ∼, collagen-like regions of the molecule proposed to be in a triple helix (Reid & Porter, 1976). (*a*) Intact subcomponent C1q; (*b*) pepsin-digested subcomponent C1q; (*c*) the large pepsin fragment of subcomponent C1q after heat denaturation, C1q has been shown to be composed of nine disulphide-linked dimers, i.e. six A-B dimers plus three C-C dimers (Reid & Porter, 1976); (*d*) heat-denatured pepsin fragment of subcomponent C1q that had recovered approx. 60% of its original collagen-like structure as estimated by c.d.

two of the flexible connecting strands of the molecule (Fig. 5).

Discussion

C.d. spectroscopy has been used as an empirical tool for the determination of the structure of proteins and polypeptides in solution, since it gives characteristic peptide bands in the far-u.v. region for different conformations (Table 3; Fasman, 1973). The small positive band at 230nm in the c.d. spectrum of whole subcomponent C1q (Fig. 1 and Table 1) suggests the presence of some collagen-like structure, since no other native polypeptide conformation would be expected to show a positive peak in this region (Table 3). The only non-collagen conformation that has a positive band in the 215–230nm region is that found for charged polypeptides, e.g. polylysine at low pH, which shows a positive band at 217nm (of about +5000) and a negative band near 198nm (of about −10000 to −20000). There is considerable debate as to whether such polypeptides are in some locally extended form, as argued convincingly by Krimm & Tiffany (1974), or in a totally disordered state. It seems unlikely that the C1q positive band is related to such charged polypeptide effects because of the specific effects of pepsin and collagenase on the band (Fig. 4). However, the low magnitude and high wavelength of this band compared with that found for lathyritic rat skin collagen (Table 1) and the low magnitude of the negative band near 200nm suggest that this spectrum could be a composite of some other structure, such as β- or α-helix, with a collagen-like one.

Table 3. *C.d. features obtained for the different types of conformations found in proteins and polypeptides in solution*

The magnitudes are given as mean residue ellipticities and are taken from Fasman (1973), Krimm & Tiffany (1974) and Tiffany & Krimm (1969).

Conformation	C.d. features	
	nm	Magnitude
α-Helix	193	+73000
	208	−35000
	222	−38000
β Structure	198	+50000
	217	−8000
Collagen	198	−50000
	220	+6000
'Random' form heat-denatured collagen	200	−15000
Charged polypeptides 'Extended form'	198	−200000 to −40000
	217	+5000

After digestion of subcomponent C1q with pepsin, the positive band is shifted to 223nm, which is more typical of collagen (220nm) and has a higher magnitude (Table 1), which is consistent with the pepsin fragmenting the non-collagenous portions of the molecule to small peptides (Reid, 1976). The amino acid-sequence data on the pepsin fragment of subcomponent C1q suggest that about 84% of the residues are in the form of three different regions of Gly-X-Y repeating triplet sequence (Reid, 1976), yet the magnitudes of both extrema are considerably less (one-quarter at 200nm, one-half at 223nm) than that expected for unaggregated collagen molecules of 80% triple-helical structure (Table 1). If the 15–20% of the residues in the pepsin fragment that do not have collagen-like sequences are in an α-helical or β conformation, this could in large part explain the low ellipticity observed at 223nm, since these structures show a high negative mean residue ellipticity at this wavelength (Table 3). Aggregation of the triple helices, thought to be present in subcomponent C1q, could also have some effect on the magnitude, since it has been found that aggregated α-helices give a considerably lower magnitude (and a longer wavelength position) for the c.d. minimum near 222nm than that found for unaggregated α-helices (Ji & Urry, 1969).

The thermal-transition temperatures of subcomponent C1q and the pepsin fragment of subcomponent C1q were about 10°C higher than that observed for lathyritic rat skin collagen (Table 2), which was surprising considering the length of the collagen-like regions (78 residues each) and the relatively low imino acid content (14%) of one of the regions, although it should be noted that the other two regions have imino acid contents of 23 and 26% (Reid, 1976). It is possible that aggregation of the six triple helices postulated to be present in subcomponent C1q (Reid & Porter, 1976) stabilizes the molecule against denaturation by heat, since aggregation of collagen molecules into fibres is known to have a stabilizing effect against heat denaturation (Piez & Gross, 1960). Also consistent with the suggestion that any triple-helical structure in subcomponent C1q might be more resistant to heat than that found in collagen are the following observations: pepsin digestion of human subcomponent C1q can be performed at 37°C, which leaves the entire regions of collagen-like sequence intact (Reid, 1976); rabbit subcomponent C1q was found to retain all its haemolytic activity (and presumably structure) after heating, at pH7.4, up to a temperature of 48°C, whereas at 50°C only 45% of the activity remained, after cooling, and at 52°C, no activity was recovered on cooling (Lowe & Reid, 1974). The reduced and alkylated pepsin fragment of subcomponent C1q had a lower thermal-transition temperature than the whole subcomponent C1q or the unreduced pepsin frag-

ment (Table 2), which suggests that the interchain disulphide bonds at the *N*-termini of each of the three collagen-like regions (Reid, 1976) may also enhance the stability of any triple-helical type structure in subcomponent C1q, or it may be that alkylation of the cysteine residues at A4, B4 and C4 has disrupted the structure to some extent.

Only the unreduced pepsin fragment of subcomponent C1q appeared to regain a significant amount of collagen-like structure after heating then cooling again as estimated by the re-formation of the positive band at 223nm in the c.d. spectrum. The electron-microscopy studies also indicated that this fraction was the only one to show particles that bore any resemblance to the unheated material, and in this sample the renatured material was composed of rod-like shapes (2–3nm×20–30nm), each of which could be interpreted as being composed of two of the connecting strands lined up in parallel and joined to a region that corresponds to one-third of the central fibril-like portion of the intact molecule (Fig. 5). One might have expected intact unreduced subcomponent C1q to renature, and perhaps to reaggregate, but its failure to do so could be due to irreversible denaturation taking place in the globular regions on heating. It is not clear why reduction and alkylation of the pepsin fragment of subcomponent C1q should interfere with renaturation, since the disulphide bonds in this fragment connect two dimers of polypeptide chains, at the very beginning of the collagen-like sequence for the C-C dimer and just before the start of the collagen-like sequences for the A-B dimer (A, B and C being the three types of polypeptide chain found in subcomponent C1q, see Fig. 5 and Reid, 1976). This suggests that there may be some role for these disulphide bonds in triple-helix formation. It is noteworthy that when disulphide-linked *Ascaris* collagen is reduced and carboxymethylated, it has a significantly lower 'melting' temperature and much decreased ability to renature than either the native or reoxidized molecule (McBride & Harrington, 1967). The recent observations that the collagen precursor (type 1 collagen) has disulphide bonds in its *N*-terminal portion again suggests a possible role for such covalent bonding in helix formation.

It has been established by previous electron-microscopy studies (Shelton *et al.*, 1972; Knobel *et al.*, 1975) that subcomponent C1q is composed of six peripheral globular regions (5nm×7nm, each) each joined by a connecting strand (1.5nm×11.5nm) to a fibril-like central portion (4.5nm×11.2nm) (Fig. 5). These observations were confirmed (Plates 1*a* and 2), and it was further shown that the pepsin fragment of subcomponent C1q was composed only of molecules that had the six connecting strands and fibril-like central portion (Plates 1*b* and 2), thus confirming that the six peripheral globular regions were fragmented by pepsin to yield small peptides.

These electron-microscopy and c.d. studies taken in conjunction with the amino acid-sequence studies on the pepsin fragment of subcomponent C1q have led to the proposal that subcomponent C1q is composed of three disulphide-bonded pairs of units, each unit being composed of one of each of the A, B and C chains of subcomponent C1q (Reid & Porter, 1976). It was further proposed that the collagen-like sequences of 78 residues in each chain may form a collagen-type triple helix, half of which may correspond to one connecting strand and the other half to one-sixth of the fibril-like central portion.

The complete and rapid (approx. 1min) loss of collagen-like structure, as estimated from c.d. studies, by the pepsin fragment of subcomponent C1q on digestion with bacterial collagenase was surprising in view of the slow (15–20h) loss of collagen-like structure by the intact subcomponent C1q under the same digestion conditions. The difference in the rates of digestion could be explained by one of the following reasons: the pepsin fragment lacks the six peripheral globular regions, which are fragmented by pepsin, and it may be that the collagen-like regions are more exposed than they are in the intact molecule and therefore more susceptible to attack by collagenase, since there would be no possibility of steric hindrance by the globular regions; the *C*-termini of the collagen-like regions of the pepsin fragment, which are exposed by pepsin digestion, have, unlike the *N*-termini, no interchain disulphide bonds (Reid, 1976) and this may be of importance in obtaining rapid digestion by collagenase. Lathyritic rat skin collagen contains no disulphide bonds and is digested rapidly by collagenase, but it will probably be of greater interest to compare the rates of digestion, by collagenase, of intact subcomponent C1q and pro-collagen (which has non-helical *N*- and *C*-terminal extensions containing disulphide bonds).

In conclusion, it appears that subcomponent C1q contains some collagen-like triple-helical structure that is enriched on pepsin treatment and lost by collagenase digestion. There certainly appears to be some other structure in addition to collagen present in subcomponent C1q and although the c.d. curves would be consistent with either β- or α-helical structure, it is difficult to say which from this technique. It is noteworthy that some of the physical properties observed may be due to the aggregation of the triple helices into a hexamer, e.g. the high thermal-transition temperature, the very low rate of collagenase digestion of the intact subcomponent C1q and perhaps some of the low magnitudes of the c.d. spectra.

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